Structural Lipidomics and Molecular Imaging of C=C Positional Isomers Enabled by mCPBA Epoxidation with Tandem Mass Spectrometry

Ting-Hao Kuo,[1] Hsin-Hsiang Chung,[1] Hsin-Yuan Chang,[1] Chiao-Wei Lin,[2,3] Ming-Yang Wang,[4] Tang-Long Shen,[3] Cheng-Chih Hsu[1*]

[1] Department of Chemistry, [2] Department of Animal Science and Technology, [3] Department of Plant Pathology and Microbiology, National Taiwan University, Taipei 10617, Taiwan

[4] National Taiwan University Hospital and College of Medicine, Taipei 100, Taiwan

ABSTRACT: Biosynthesis of unsaturated lipids is highly regulated through cellular lipogenesis, rendering diverse double-bond positional isomers (C=C isomers) of a given unsaturated lipid species. However, identification and quantification of C=C isomers are usually challenging using conventional mass spectrometric analyses as they yield indistinguishable tandem mass spectra. To address this challenge, we proposed an easy-to-use, cost-effective, handy derivatization method, **MELDI** (**m**CPBA **E**poxidation for **L**ipid **D**ouble-bond **I**dentification), and its integrations with liquid chromatography mass spectrometry (LC-MS) lipidomics as well as desorption electrospray ionization (DESI) mass spectrometry imaging (MSI). Using MELDI-LC-MS, we identified more than 100 C=C isomers among mono- and poly-unsaturated fatty acids and glycerolphospholipids in human serum, where a variety of uncommon C=C isomers with low endogenous levels were quantified. The capability of MELDI-LC-MS in lipidome analysis was also demonstrated by the differentiated 3T3-L1 adipocytes, providing an insight into the cellular lipid reprogramming upon stearoyl-coenzyme A desaturase 1 (SCD1) inhibition at the C=C isomer level. Additionally, combining MELDI with DESI-MSI allowed us to spatially resolve the tumor-associated change in C=C isomers in a metastatic mouse lung tissue section. Our results suggested that C=C isomers are potential indicators to disease-related alterations in cellular lipid homeostasis.

INTRODUCTION

Lipidomics has emerged as a distinct discipline that can elucidate biochemical processes involved in lipid metabolism.[1] This field has been driven by modern mass spectrometry (MS) technologies, whereas lipid classes and species, i.e. the length and degree of unsaturation of individual fatty acyl chains, can be routinely determined by tandem mass spectrometry (MS/MS).[2] Nonetheless, a long-standing challenge of lipid characterization is to resolve double-bond positional isomers (C=C isomers) among unsaturated lipids. These C=C isomers are often indistinguishable by conventional MS/MS, *e.g.* collision-induced dissociation (CID), as the isomers yield near-identical fragments,[3] leading to obscure assignment of C=C positions in a vast majority of lipidomic studies.[4-5] Such ambiguity of lipid structures restricts the mining of lipid biology as each C=C isomer represents a distinct pathway regulated by enzymatic reactions in cellular lipogenesis.[6]

In the most recent years, there have been a booming development of MS-based methods to address this challenge. These methods can roughly be summarized into two approaches. The first one utilizes specialized ion activation in the custom-made mass spectrometers so as to generate distinct diagnostic product ions indicative to the C=C positions. These methods, including ultraviolet-photodissociation (UVPD),[7-10] electron impact excitation of ions from organics (EIEIO)[7,11-12] and ozone-induced dissociation (OzID),[13-15] have been applied to characterize C=C positions of fatty acyl chains in fatty acids (FAs)[14] and glycerophospholipids (GPLs).[7-8,10] More recently, both UVPD and OzID have been coupled with mass spectrometry imaging (MSI) to provide the C=C isomers distribution on biological tissue sections.[10,15] Ion-mobility spectrometry (IMS) and liquid chromatography (LC) allow to separate C=C isomers to some degree and have been coupled with OzID to resolve the C=C positions.[16-18] However, the accessibility of the specialized ion activation techniques remains limited to reach to a larger lipidomics society.

Another approach is to remove the double bond nature of the C=C isomers by chemical derivatization of the sample. Such chemical derivatization-based methods include ozonolysis,[19-21] *Paternò–Büchi* (P-B) photochemical reaction[22-29] and low-temperature plasma-induced epoxidation,[30-32] charge-switch derivatization.[33] The subsequent modified versions of the C=C isomers are usually labile and their C=C positions can thus be determined by CID to generate the diagnostic ions, except for ozonolysis that C=C bonds are cleaved for identification. In addition, most of the methods were implemented with shotgun lipidomics,[19-20,22-25,30-33] where lipids are directly ionized through electrospray ionization (ESI) or nano-ESI without separation. Notably, on-tissue (offline) P-B reaction using a reaction chamber was also demonstrated, which allowed C=C

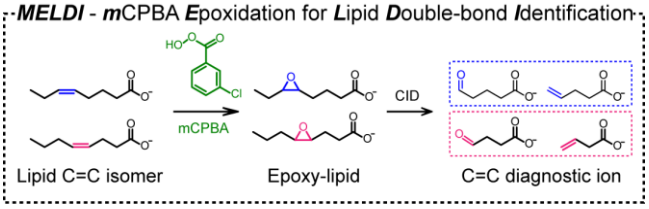
isomeric MSI.[27] Furthermore, online P-B reaction can be coupled with ambient ionization for rapid *in-situ* profiling of C=C isomers in animal tissue sections.[26,29] More recently, the combination of online P-B reaction with LC-MS/MS has been demonstrated for large-scale analysis of C=C isomers.[28] Nonetheless, these derivatization-based methods still require either modification on ionization sources or particular equipment to carry out lipid derivatization.

The above C=C isomer-resolving methods have expanded the boundary of lipidomics. Using these methods, unsaturated lipids of C=C isomeric mixtures have been profoundly investigated in many biological systems.[10,15,23,26,28] In addition, screening of C=C isomeric composition changes has been proposed for biomarker discovery.[28] Bearing these in mind, we were thus motivated urgently to develop a method with universal accessibility, so as to make the study of C=C isomers in a versatile and handy fashion. Here we describe such an epoxidation derivatization-based method, **MELDI** – (**m**CPBA **E**poxidation for **L**ipid **D**ouble-bond **I**dentification) and its utilization in LC-MS-based lipidomics and mass spectrometry imaging. More specifically, MELDI utilizes the derivatization reagent, *meta*-chloroperoxybenzoic acid (mCPBA), which has been profoundly used in many organic synthesis procedures to efficiently generate epoxides on C=C in organic solvents.[34] Subsequently, the affirmative C=C position diagnosis is easily achieved with CID, one of the most commonly used MS/MS fragmentations provided by commercial mass spectrometers. Recently, Li's group has showed the exceptional utilities of mCPBA epoxidation as it provides high epoxidation specificity to C=C bonds, a fast derivatization that can be achieved in minutes, abundant MS/MS fragments for C=C position assignments, and applicability in multiple lipid classes.[35] In addition, they have also demonstrated the combination of MELDI with shotgun analysis to identify C=C isomers in a yeast lipid extract in positive ionization mode. In the present study, we will demonstrate the wide versatility of MELDI employed with LC-MS and MSI in biological samples for the first time. In short, we showed that (i) identification of more than a hundred of C=C isomers in unsaturated fatty acids (FAs) and glycerophospholipids (GPLs) in human serum lipid extracts and subsequent quantification of a variety of uncommon low-abundant C=C isomers, (ii) the alterations of C=C isomer compositions in the differentiated 3T3-L1 adipocytes induced by the inhibition of stearoyl-coenzyme A desaturase 1 (SCD1), and (iii) imaging of the tumor-associated changes in C=C isomers on a metastatic mouse lung tissue section. These results suggested that C=C isomers are important to cellular lipid homeostasis and thus serve as potential disease biomarkers.

Results and Discussion

Pinpoint C=C positions in unsaturated lipids by MELDI-LC-MS/MS.

The scheme of MELDI is sketched in **Scheme 1**. In MELDI, a common epoxidation reagent in organic synthesis, mCPBA, is used to derivatize C=C bond into an epoxide prior to ESI-MS analysis in negative ionization mode. In the subsequent MS/MS, the epoxide is labile upon CID, generating an aldehyde/alkene diagnostic ion pair to indicate the original C=C position.



Scheme 1. MELDI (mCPBA Epoxidation for Lipid Double-bond Identification) to pinpoint C=C positions in unsaturated lipids. In MELDI, a C=C bond of an unsaturated lipid is epoxidized by mCPBA to form an epoxide, which is labile upon CID, so as to yield a diagnostic ion pair for indication of the original C=C position.

The utility of MELDI to pinpoint C=C positions was systematically investigated using a series of commercial standard mono-unsaturated lipids by high-resolution Fourier transform (FT) MS/MS spectra. First, we showed that for two monounsaturated fatty acid (MOFA) C=C isomers, FA 18:1 (9Z) and FA 18:1 (11Z), after in-solution epoxidation, distinguishable CID spectra of their dehydrated parent ions ($[M-H]^-$ = *m/z* 297.2435) were obtained (**Fig. 1a and 1b**). Their C=C positions were identified by the abundant C=C diagnostic ion pairs, (*m/z* 155.1077 and 171.1025) and (*m/z* 183.1391 and 199.1339) for Δ9 and Δ11, respectively. The epoxide ring breaking upon CID was also confirmed by a deuterium labeled standard, D_{17}-FA 18:1 (9Z) (**Fig 1c**). For another MOFA, FA 16:1 (9Z), with a shorter carbon chain and a C=C bond at Δ9, the CID spectrum of its epoxide showed an identical diagnostic ion pair at *m/z* 155.1077 and 171.1025 as in FA 18:1 (9Z) (**Fig. 1d**). In addition to FAs, the C=C position of MOFA in a GPL molecule can be similarly identified through sequential CID (CID/CID) of the epoxy-FA daughter ion in the MS/MS spectra. Such strategy was further demonstrated by various subclasses of GPLs, including phosphatidylethanolamine (PE) (**Fig. 1e**), phosphatidylserine (PS) (**Fig. S1a**), phosphatidylcholine (PC) (**Fig. S1b**), phosphatidic acid (PA) (**Fig. S1c**). In summary, the C=C diagnostic ions generated from MOFA-derived epoxides are predictable and merely follow Δ positions regardless of fatty acyl chain lengths.

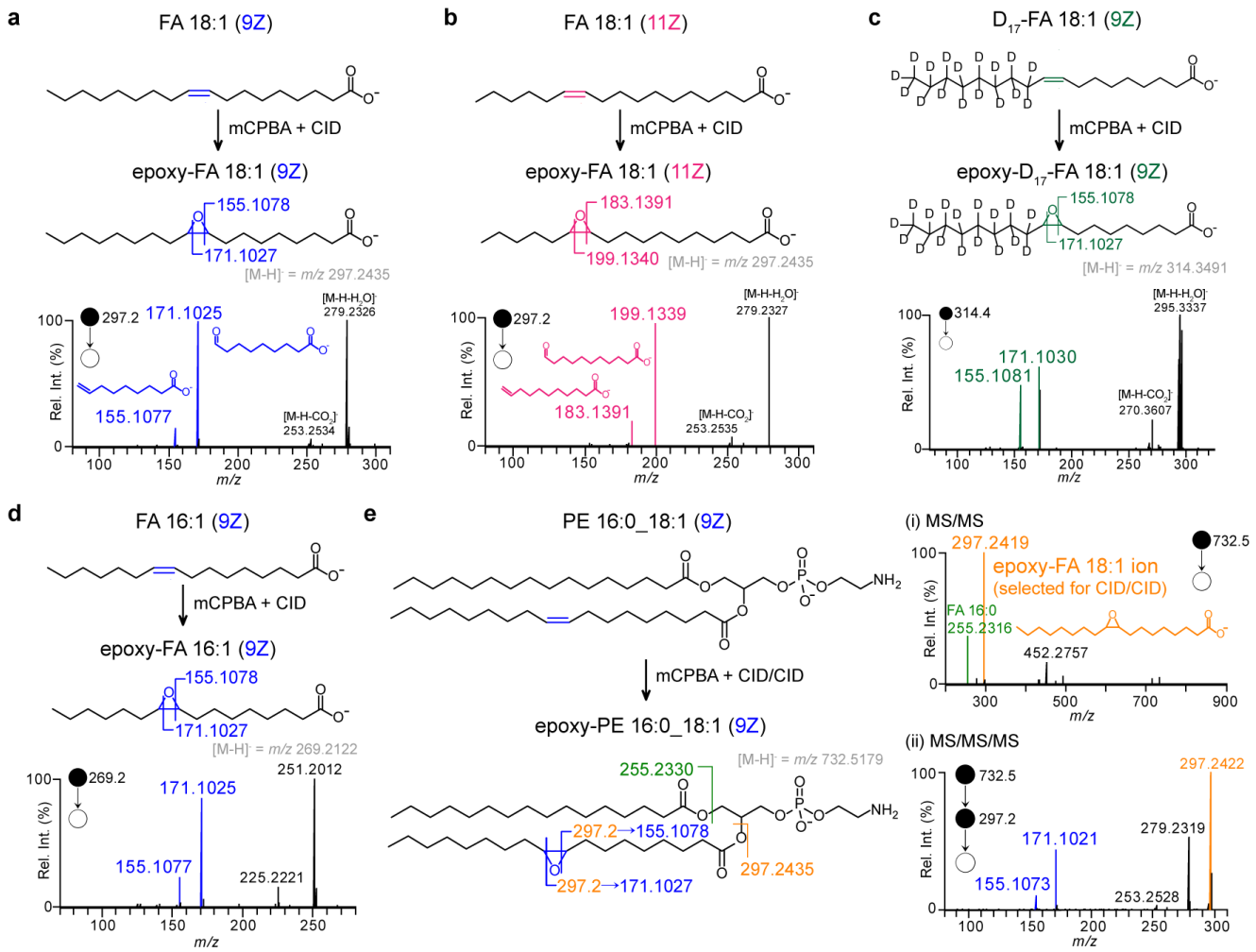


Figure 1. Pinpointing C=C positions in monounsaturated lipids with MELDI-MS/MS. The FT-MS/MS spectra and CID schemes of **(a)** FA 18:1 (9Z) **(b)** FA 18:1 (11Z) **(c)** D_{17}-FA 18:1 (9Z) **(d)** FA 16:1 (9Z), where the C=C diagnostic ion pairs generated upon CID of the epoxidized lipid were indicated. **(e)** For PE 16:0_18:1 (9Z), its C=C position was elucidated by MS^3 of the epoxy-FA 18:1 acyl chain ion (*m/z* 297.24) from the MS/MS spectrum of the epoxidized lipid parent ion (*m/z* 732.52).

Notably, in addition to allowing the CID-based diagnosis of C=C positions, mCPBA epoxidation also enhanced chromatographic separation of unsaturated lipids in reverse-phase (RP) LC. This was demonstrated by an isomeric mixture of equal amount of FA 18:1 (9Z) and FA 18:1 (11Z) (100 µM for each). As shown in **Fig 2a**, the FA 18:1 C=C isomers were not separated in RPLC-MS before derivatization, as shown by a co-eluted peak in the extracted ion chromatogram (XIC) of the dehydrogenated parent ion of FA 18:1 ($[M-H]^-$ = *m/z* 281.2475). The same isomeric mixture was then derivatized with excess mCPBA (200 mM) in organic solution, and incubated at 50 °C for an hour to reach to the optimal reaction yield before it was analyzed with LC-MS/MS. As a result, most of the non-derivatized FA isomers were converted into the epoxides, as shown by two distinguishable peaks in the XIC of epoxy-FA 18:1 (**Fig. 2b**), allowing subsequent MS/MS analysis to determine their C=C positions. The result implied (i) the epoxide yields of 9Z and 11Z isomers were almost the same, referred by their similar peak area, (ii) epoxidation of an unsaturated lipid increased its hydrophilicity, resulting in the earlier elution in RPLC (iii) difference in the affinity with the column between C=C isomers was enhanced after epoxidation, as indicated by two separated peaks. On the other hand, for epoxidized geometric isomers in either E- or Z-conformation (*e.g.* epoxy-FA 18:1 (9Z) and epoxy-FA 18:1 (9E)), though could be separated in RPLC, their MS/MS spectra were indistinguishable (**Fig. S2**). Therefore, C=C positions are annotated in Δ-nomenclature without referring the C=C geometry throughout this study.

Ideally, MELDI could be applied to the diagnosis of multiple C=C positions of a polyunsaturated fatty acid (PUFA) using similar approaches. However, we found that when two or more epoxides were tagged in a PUFA, its MS/MS spectra became complicated, making it difficult to probe the C=C diagnostic ions (**Fig S3**). In this regard, many shotgun lipidomic approaches thus aimed for the MS/MS analysis of the singly tagged PUFA.[23-24,30-31] However, as multiple mono-epoxidized products of the same *m/z* were

unable to be differentiated in a shotgun approach, its MS/MS analysis is still challenging especially when the numbers of C=C bonds are large.

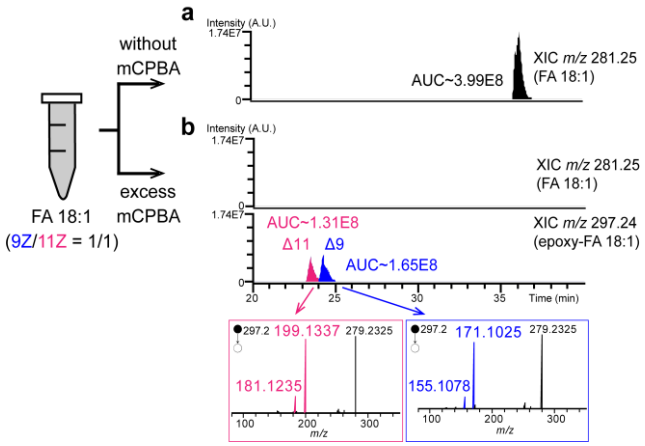


Figure 2. MELDI-LC-MS/MS analysis of FA 18:1 C=C isomers. The FA 18:1 C=C isomer mixture (9Z/11Z = 1/1) was analyzed by LC-MS/MS **(a)** prior to mCPBA epoxidation and **(b)** after derivatization with excess mCPBA. The extracted ion chromatograms (XIC) of FA 18:1 and epoxy-FA 18:1, and the area under curve (AUC) were shown.

To solve this issue, we demonstrated that coupling MELDI with LC-MS provided an effective way to confidently identify C=C positions in PUFAs. Herein a series of standard PUFAs were thoroughly investigated by MELDI-LC-MS/MS. First, taking FA 18:2 (9Z, 12Z) for example, when moderate epoxidation was conducted, two mono-epoxidized products were found, separated at different retention time and thus distinguished at MS1 level. Subsequent MS/MS provided further structural details by revealing the Δ9 and Δ12 double bond nature (**Fig. 3a**). Applying a similar approach to ω-6 FA 18:3 (6Z, 9Z, 12Z), three mono-epoxidized products of C=C bonds at Δ6, Δ9, or Δ12, were revealed (**Fig. 3b**). Another common FA 18:3 C=C isomer, ω-3 FA 18:3 (9Z, 12Z, 15Z), was also studied, giving a LC-MS/MS spectral set of three epoxy-FA 18:3 products (**Fig. 3c**), which was obviously distinguishable from that of the ω-6 isomer. More cases of PUFA, including FA 20:2, FA 20:3, FA 20:3, FA 20:4, and FA 20:5, were individually investigated and provided in **Fig S4**. It is noteworthy that, in comparison to MOFAs, the CID fragmentation of mono-epoxidized products of PUFAs was more sophisticated. In specific, the CID fragmentation favors the daughter ions of the cleavages at the sides adjacent to the other unsaturated sites in the epoxides. Such tendency may be due to the generation of the products with conjugated π-bond systems upon CID (**Fig. S5**). In addition, we also noted that in some cases the fragmentation occurs at the adjacent carbon-carbon bond especially when the epoxide positions were within eight carbon atoms to the carboxyl end. For a poly-unsaturated GPL, C=C positions in its PUFA chain could be similarly resolved by LC-MS^3 of its mono-epoxidized products, in which CID/CID was applied to the epoxy-PUFA daughter ion in the MS/MS spectrum (**Fig. S6**).

Importantly, the epoxidation using mCPBA was highly specific to C=C bonds,[34-35] resulting in appreciable epoxide yields for unsaturated lipids (**Fig. S7, Table S1**). This further made MELDI-LC-MS reliable in probing C=C diagnostic ions from low amount of unsaturated FAs in the nM level, while the limit of detection (LOD) of C=C diagnostic ions for GPLs was assessed at sub-μM level due to the requirement of MS^3 (**Fig. S8-S9**).

Quantification of lipid C=C isomers with MELDI-LC-MS

Quantification of C=C isomers via MELDI-LC-MS-MS/MS analysis was validated as described in the followings, and experimental details were provided in **Supporting Information**. We first tested a series of mixtures containing two FA 18:1 C=C isomers, 9Z and 11Z, with different molar fractions. The samples were epoxidized with excess mCPBA and analyzed by targeted LC-MS/MS at *m/z* 297.24 (epoxy-FA 18:1). Then, the extracted ion chromatogram (XIC) area under curve (AUC) of two C=C diagnostic ions for each isomer at different molar fractions was acquired so as to construct the calibration curve (**Fig. 4a**). As a result, a good linearity between the relative ratios of XIC AUC of diagnostic ions ($A_{11Z}/(A_{9Z}+A_{11Z})$) and their molar fractions were obtained ($R^2 \sim 0.999$). Interestingly, the slope of the calibration curve (0.98) is close to unity, suggesting that the monounsaturated C=C isomers possess the same levels of epoxidation yields, ionization efficiency, and similar fragmentation features. Such characters are advantageous in determining the relative proportion of each C=C positional isomer in a given unsaturated lipid species, especially when the commercial standards are not available. For absolute quantification of FA 18:1 isomers, D_{17}-FA 18:1 (9Z) was spiked into the samples and used as the internal standard (IS) to quantify FA 18:1 (Δ9) and FA 18:1 (Δ11) (**Fig. 4b**).

Quantification of PUFA C=C isomers was also validated using a series of ω-3 and ω-6 FA 18:3 isomer mixtures in a similar way, while moderate epoxidation condition was applied to yield high amount of the mono-epoxidized (**Fig S10, Table S2**). **Fig. 4c** showed the obtained calibration curve, which also had a good linearity ($R^2 \sim 0.999$). This enabled the determination of the endogenous ω-3/ω-6 isomeric ratio of FA 18:3 in human sera (94.3 % / 5.7 %, SD = 3.0 %; n = 7). Finally, quantifications of ω-6 and ω-9 FA 20:3 mixtures were also validated (**Fig. 4d**).

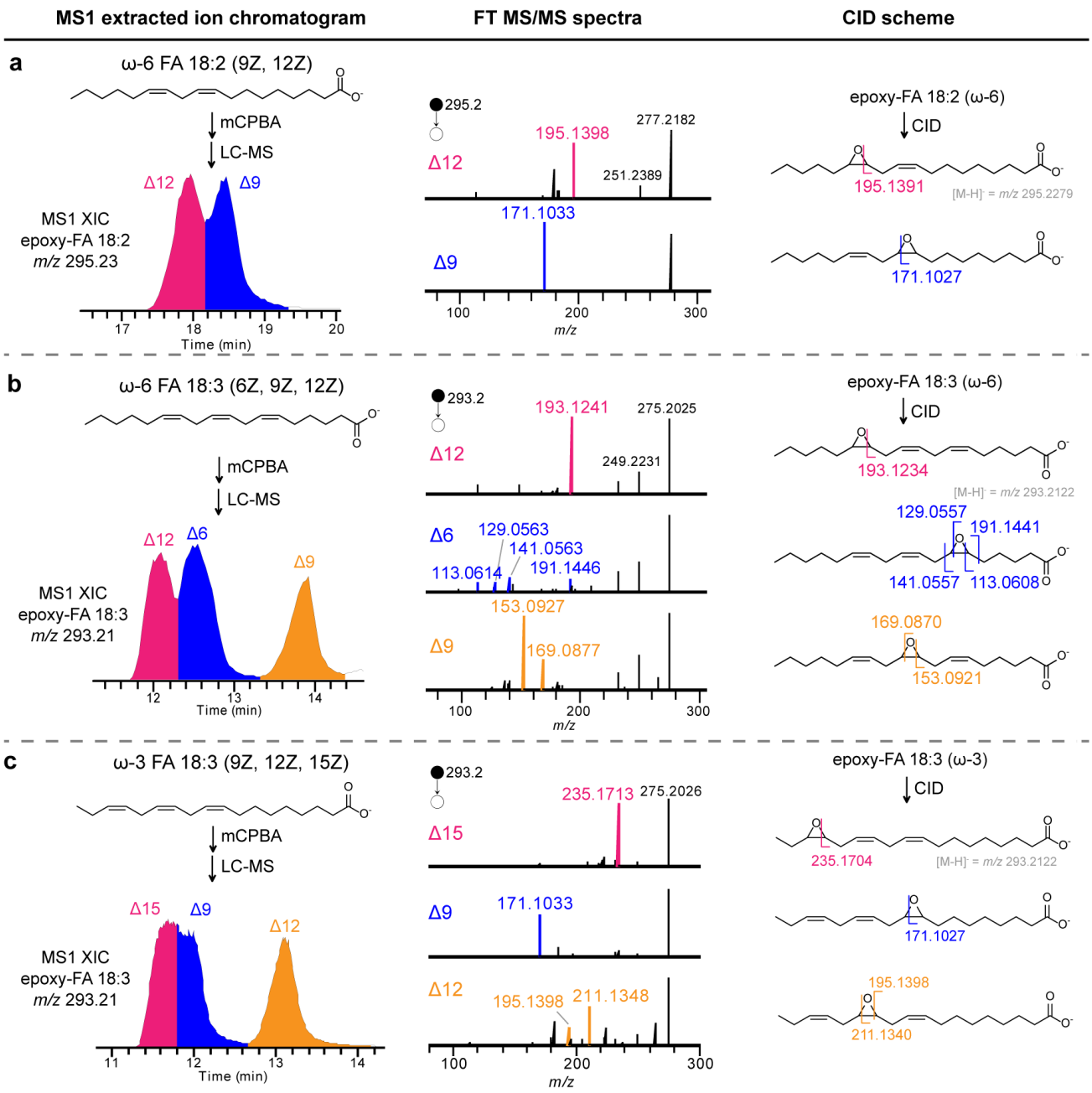


Figure 3. Identification of multiple C=C positions of polyunsaturated lipids by MELDI-LC-MS/MS. The MELDI-LC-MS/MS analysis of polyunsaturated lipids, including **(a)** ω-6 FA 18:2 (9Z, 12Z), **(b)** ω-6 FA 18:3 (6Z, 9Z, 12Z), and **(c)** ω-3 FA 18:3 (9Z, 12Z, 15Z), are demonstrated. Multiple C=C positions of each polyunsaturated lipid are subsequently identified by the MS/MS spectral set of its mono-epoxides.

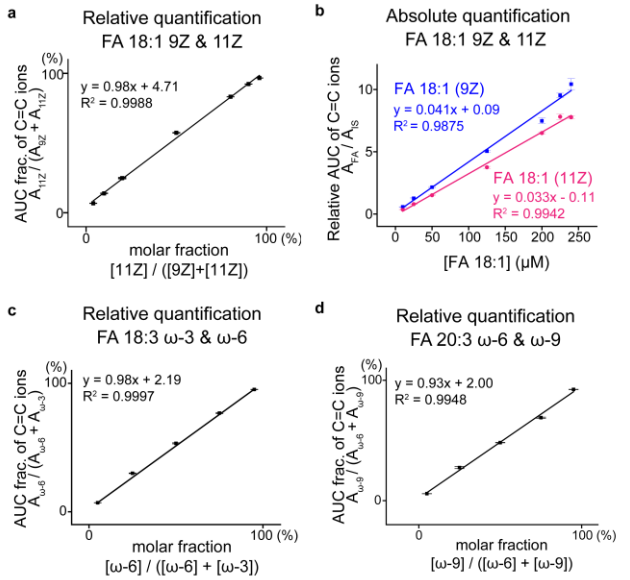


Figure 4. Quantification of lipid C=C isomers by MELDI-LC-MS/MS. The calibration curves for relative quantifications of **(a)** FA 18:1 (9Z) and (11Z) isomers **(c)** FA 18:3 ω-3 and ω-6 isomers **(d)** FA 20:3 ω-6 and ω-9 isomers. **(b)** The calibration curve for absolute quantification of FA 18:1 C=C isomers, where D_{17}-FA 18:1 (9Z) was used as internal standard (IS). Each point represents mean ± SD calculated from a triplicate. The experimental details were described in **Supporting Information**.

Coupling MELDI with LC-MS-tPRM for identification and quantification of lipid C=C isomers in biological systems

LC-MS-based lipidomics provide high sensitivity and lipid coverage as the matrix effect is reduced by chromatographic separation, but yet the information of C=C isomers are still obscure in many lipidomics reports.[4-5] To address such structural ambiguity, we integrate MELDI with triggered parallel reaction monitoring (tPRM)[36] as an innovative method for semi-targeted analysis of C=C isomers using LTQ-Orbitrap mass spectrometer. The concept of LC-MS-tPRM analysis is introduced in **Fig. 5a**. In LC-MS-tPRM, it continuously acquires full FT-MS spectra when analytes are eluting, and once the pre-defined targeted precursor ions (*i.e.* mono-epoxidized lipids) are detected, the corresponding MS/MS events will be triggered in each scanning cycle (*e.g.* the most N intense precursor ions are selected for MS/MS acquisition in a top-N tPRM method). In fact, tPRM can be regarded as a modified data-dependent acquisition (DDA), in which the targeted precursor list is pre-defined and the precursor ion exclusion is turned off. In comparison to multiple reaction monitoring (MRM), which measures a limited number of targeted daughter ions at narrow spectral windows, tPRM simultaneously measures all daughter ions generated in a full MS/MS spectrum. More importantly, such feature of tPRM is advantageous to acquire multiple C=C diagnostic ions from each epoxy-lipid with minimal matrix effect, allowing accurate identification and quantification of C=C isomeric mixtures.

In order to implement MELDI into the current pipeline of LC-MS-based lipidomics, we developed a two-stage workflow by integrating the conventional LC-MS lipidomics with parallel MELDI-LC-MS-tPRM analysis (**Fig. 5b-c**), as a complementary way for semi-targeted analysis on C=C isomer compositions. In the first stage, as shown in **Fig. 5b**, untargeted LC-MS/MS analysis of biological lipid extract is executed, and the resulting data was processed by LipidSearch (Thermo) to give a list of identified lipid species including putative structural information of fatty acyl chain composition, e.g. length and number of C=C bonds of each fatty acyl chain. The report is then transformed into a list of predicted epoxide ions by EpoxyFinder, a lab-built graphic-user-interface (see **Supporting Information**). EpoxyFinder generates a series of suggested MS settings, including precursor ion *m/z* and CID values for the mono-epoxidized lipids. To ensure the optimal performance of the analysis, unsaturated FAs and GPLs of high abundance were specifically targeted using an intensity filter in EpoxyFinder, and a top-25 tPRM method (maximum cycling time ~ 0.1 minute) was applied throughout the following studies. In the second stage (**Fig. 5c**), MELDI-LC-MS-tPRM analysis is applied to examine the targeted unsaturated lipids in the epoxidized sample. Details of the LC-MS-tPRM data processing procedures were elaborated in **Supporting Information (SI section 3.3)**. In addition, two individual derivatization protocols were used for monounsaturated and polyunsaturated lipids respectively, as only mono-epoxidized products were targeted for MS/MS (see **Fig. S11-S12)**.

As a pilot study, MELDI-LC-MS-tPRM analysis was applied to the lipid extracts of human sera, where 80 of the abundant unsaturated lipid species were targeted, including 44 mono-unsaturated and 36 polyunsaturated lipids species (**Table S3-S4**). Specifically, we identified 58 monounsaturated C=C isomers with C=C positions varying from Δ6 to Δ15 (**Table S5**), and 48 polyunsaturated C=C isomers (**Table S6**). The representative MS/MS spectra and chromatograms were provided in **Fig. S13-S21**. Relative proportions of the C=C isomers of 18 monounsaturated lipid species were further quantitatively determined in 10 healthy (non-cancerous) human sera, as showed in **Fig. 6a**. Interestingly, we found that the absolute concentrations of FA 18:1 were diverse among the samples (141.4 ± 56.7 μM for Δ9; 18.0 ± 5.4 μM for Δ11; n = 10), while the relative proportions of C=C isomers in FA 18:1 and its constituting GPLs were quite similar (e.g. 91.8 ± 0.9 % for FA 18:1 (Δ9); 7.8 ± 0.9 % for FA 18:1 (Δ11); **Table S5**), suggesting that the C=C isomer compositions were held consistent among healthy individuals.

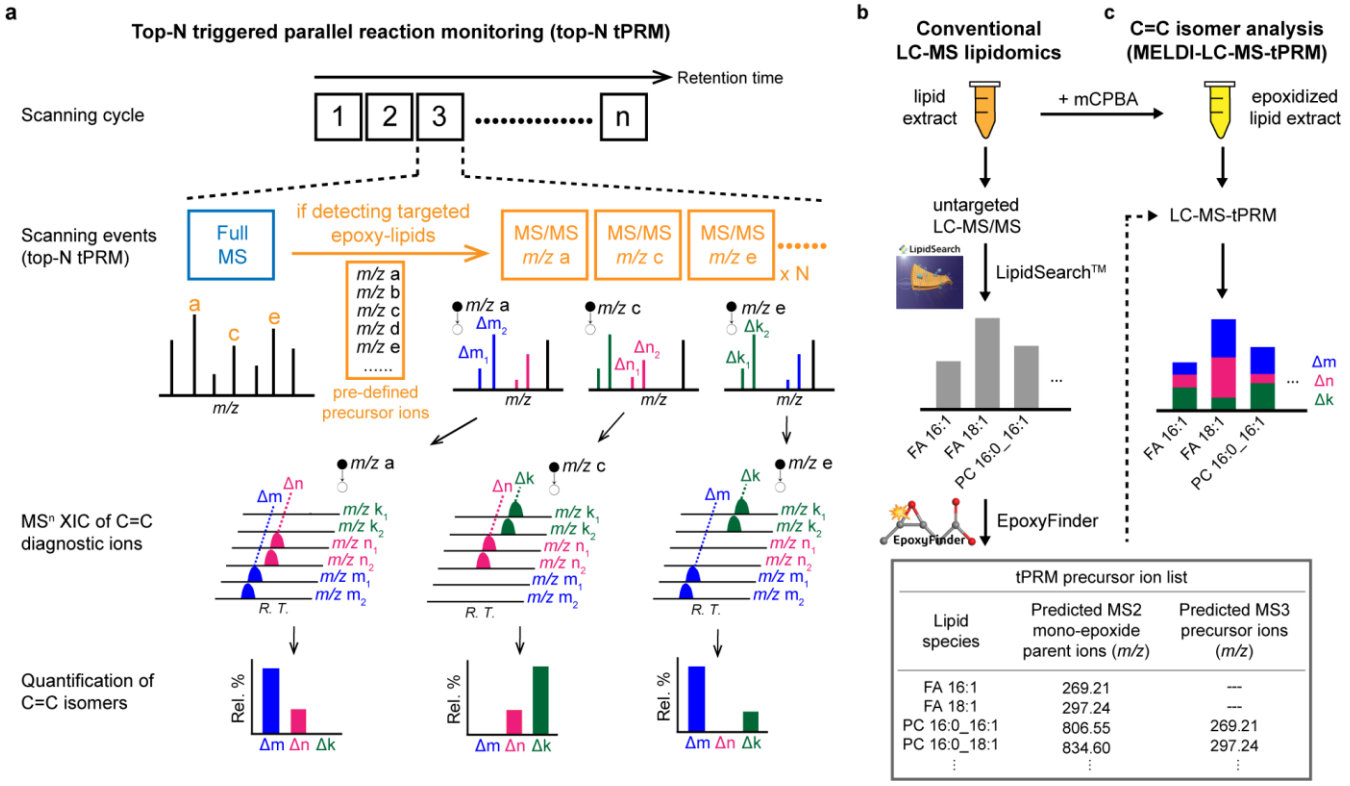


Figure 5. LC-MS-based analysis of C=C isomers enabled by MELDI-LC-MS-tPRM. (a) Schematic representation of top-N triggered parallel reaction monitoring (tPRM). In a top-N tPRM module, each scanning cycle contains one full-FTMS scan, followed by multiple IT-MS/MS (or MS^3) acquisitions of top-N most intense epoxy-lipid ions (if they are present) in the full-FTMS spectrum throughout the whole analysis. Each lipid C=C isomer is subsequently quantified by summed extracted ion chromatogram (XIC) area of a set of C=C diagnostic ions under the corresponding MS/MS scanning channel of the epoxy-lipid ion. **(b-c)** The two-stage workflow for semi-targeted C=C isomers analysis in biological lipid extracts. In the first stage in **(b)**, conventional untargeted LC-MS/MS analysis is applied to identify unsaturated lipids. The data are subsequently processed by LipidSearch™, giving structural information of unsaturated lipids in the level of fatty acyl chain compositions. Then, EpoxyFinder, a lab-built graphical-user-interface, is used to process the result to create an tPRM precursor ion list of mono-epoxidized unsaturated lipids for LC-MS-tPRM analysis of C=C isomers. In the second stage in **(c)**, the lipid extract is derivatized with mCPBA and then examined with LC-MS-tPRM for analysis of C=C isomers.

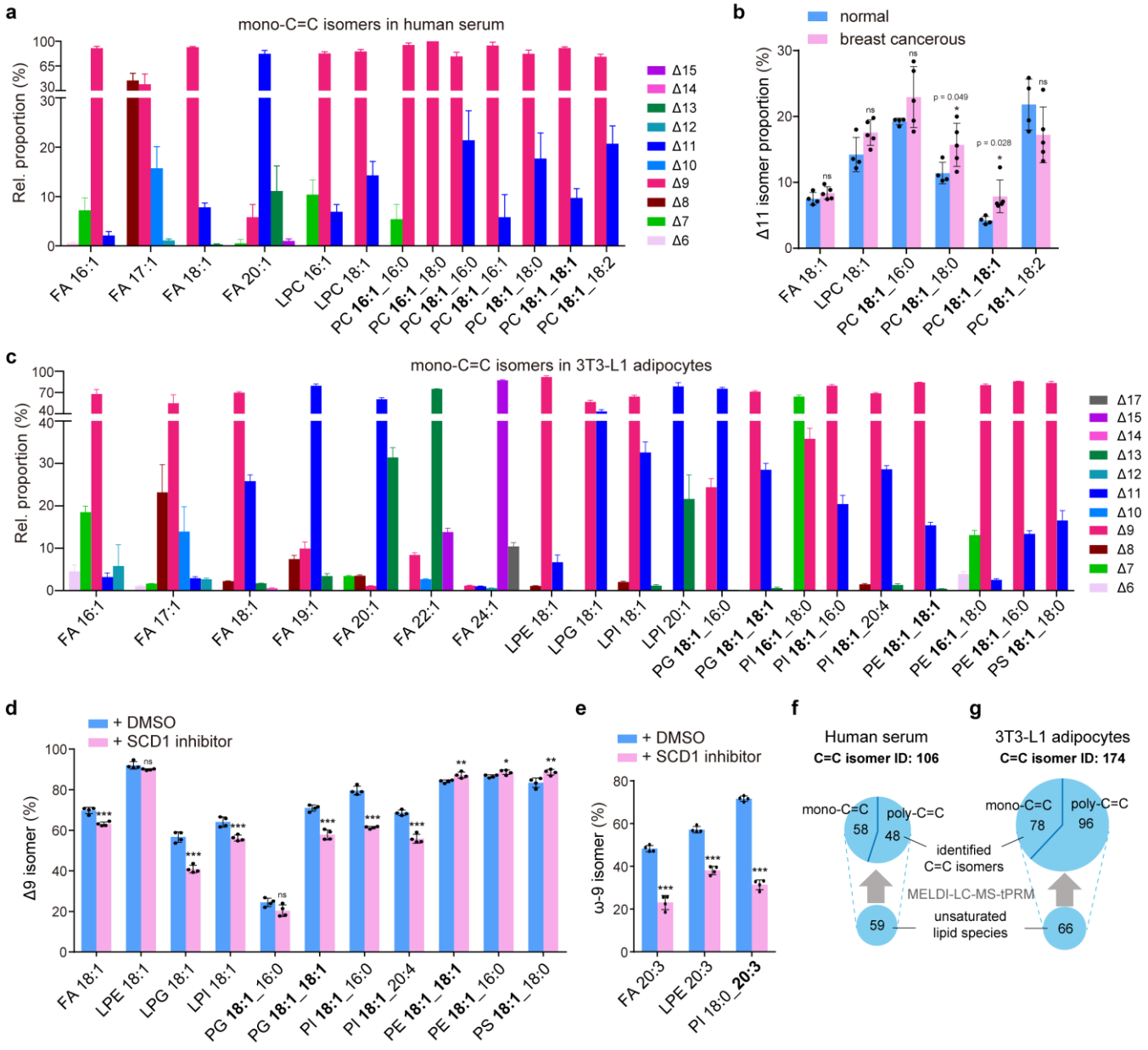


Figure 6. Lipid C=C isomers in biological systems revealed by MELDI-LC-MS-tPRM analysis. (a) Quantification of relative proportions of monounsaturated C=C isomers in the lipid extract of human serum (n = 10). **(b)** Comparison of relative proportions of Δ11 isomers in FA 18:1-containing lipids between healthy human sera (n = 4) and breast cancerous human sera (n = 5). **(c)** Quantification of relative proportions of monounsaturated C=C isomers in the lipid extract of differentiated 3T3-L1 adipocytes (n = 4). The monounsaturated FA chains in GPLs being quantified are shown in bold. Alteration of C=C isomers between normal (n = 4) and SCD1 inhibitor-treated 3T3-L1 adipocytes (n = 4) is accessed in **(d)** Δ9 isomers in FA 18:1-containing lipids and **(e)** ω-9 isomers in FA 20:3-containing lipids. Total numbers of identified C=C isomers from the targeted unsaturated lipid species in **(f)** human serum and in **(g)** 3T3-L1 adipocytes are shown as pie charts. Error bars represent SD. Statistical analyses (t test) are shown as: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$.

Furthermore, we showed that the ω-9 isomers were the most dominant species in FA 18:1, FA 20:1, FA 22:1, FA 24:1, and all the FA 18:1 chains in GPLs, which was consistent with the previous reports.[5,28,30,37] In fact, ω-9 FA 18:1 serves as the original species for the downstream biosynthesis of these ω-9 monounsaturated lipids in lipogenesis mechanism in mammalian cells.[6] In addition, the major C=C isomers of many of the PUFAs, including FA 18:2 and its biosynthetic products, FA 20:2, FA 20:3, FA 20:4, FA 22:4, and their constituting GPLs,[6] were identified as the ω-6 family (**Table S6**). These quantitative observations were consistent with the previous study on free PUFAs in human plasma,[37] and here we further confirmed the ω-6 isomers were the major C=C isomers in these PUFA-constituting GPLs. Remarkably, the presence of uncommon FA C=C isomers (*e.g.* FA 16:1 Δ6/ Δ7/Δ11; FA 18:1 Δ13; FA 20:1 Δ7/Δ9/Δ15) that are listed in LIPID MAPS database were suggested here, and their FT-MS/MS spectra were validated (**Fig. S13-S18**). These C=C isomers were of low endogenous quantities and rarely found in human serum via other C=C isomer resolving methods,[28,30] which might be due to the matrix effect as their diagnostic ions were hardly differentiated from ion interferences in complex MS/MS spectra. Here, using our platform these low-abundant C=C isomers were separated in RPLC after derivatization, and thus their relative proportions could be estimated by the AUC of diagnostic ions without sever ion interferences. To sum up, we concluded that more than 100 C=C positional isomers from 59 targeted unsaturated lipid species in human serum were identified (**Fig. 6f**).

The proposed MELDI-LC-MS platform suggested an alternative way to explore potential disease biomarkers using C=C isomer ratios.[28] We thereby studied C=C isomer compositional changes between healthy human sera and breast cancerous human sera. As a result, the proportions of the Δ11 isomer in PC **18:1**_18:2 and PC **18:1**_18:0 in the breast cancerous human serum were statistically increased (*$P < 0.05$) (**Fig. 6b and Table S7**). Our method will allow a large-scale cohort study to evaluate if such C=C isomer ratio changes can be used as cancer-related biomarkers.

We further demonstrated MELDI-LC-MS-tPRM analysis to lipid extract of the differentiated murine 3T3-L1 adipocyte, one of the most common *in vitro* models to study adipocyte biology. First, we showed that C=C isomer compositions in 20 monounsaturated lipid species were quantitatively determined (**Fig. 6c**), and the presence of low-abundant C=C isomers were supported by FT-MS/MS annotations (**Fig. S26-S32**). We concluded that the 3T3-L1 adipocytes possessed a considerable diversity of C=C isomers, rendering more than 170 C=C isomers identified in 66 unsaturated lipid species **(Fig. 6g)**. In specific**,** from the 140 targeted unsaturated lipid species (**Table S8-S9**), 77 monounsaturated C=C isomers were identified and quantified in 20 species (**Table S10**), whereas up to 96 polyunsaturated C=C isomers were identified in 46 species (**Table S11**). The representative spectra and chromatograms were provided in Fig. **S22-S31**.

To further investigate the efficacy of MELDI-LC-MS-tPRM in studying lipid biology, we further examined the alteration of C=C isomer composition in 3T3-L1 adipocytes manifested by stearoyl-coenzyme A desaturase 1 (SCD1) inhibitor treatment. In fact, SCD1 is the key enzyme that catalyzes desaturation of FA 18:0 to form FA 18:1 (9Z), which is the upstreaming compound in lipogenesis of the unsaturated lipids in ω-9 family.[6,38] Intriguingly, from the quantitative comparison of C=C isomers, we found that in the SCD1 inhibitor-treated 3T3-L1 adipocytes, (i) relative proportions of Δ9 isomer in FA 18:1 and many of the FA 18:1-containing lipids were significantly decreased (**Fig. 6d and Table S12**), and (ii) relative proportions of ω-9 isomers in FA 20:3 and its constituting lipids were significantly decreased (**Fig. 6e and Table S13**). These results illustrated how the alleviated SCD1 activity mediates cellular lipogenesis for the first time at the C=C isomer level.

Mapping alterations of lipid C=C isomers on tissue sections by MELDI-DESI

MSI techniques allow visualization of compounds in biological tissue sections, providing chemical-rich information associated with tissue heterogeneity. MSI of lipids has been largely used in tissue-typing and cancer diagnosis.[39] However, such lipid imaging can provide only the summed distribution of multiple isomeric species [40]. It has been shown that alteration of the ratios of C=C isomers can be used as makers for cancerous tissues,[10,15,23,26,28] an MSI method that can easily visualize these isomers may thus be used to determine tumor margins. Here, we reported a C=C positional isomer-resolving imaging method by coupling MELDI with desorption electrospray ionization (DESI)[41] MSI. For ease of discussion, we will refer to this integrated strategy as MELDI-DESI-MSI.

The instrumental capability in quantifying C=C isomers was evaluated (**Fig. S32-S33)**, using the intensity ratios of diagnostic ions to indicate the molar ratios of the C=C isomers. For C=C isomer imaging, high-resolution MSI (at MS1 level) was first performed in a tissue section without MELDI to obtain the lipid profile and spatial distribution, whereas its serial section was used for C=C isomer imaging. To this end, we used a simple airbrushing method to *in situ* epoxidize unsaturated lipids with mCPBA in the tissue section (**Fig S34**), which was modified from the matrix deposition method used in MALDI MSI,[42] and a demonstration video was also provided (see **Supporting Information**). Notably, the entire derivatization process took less than 10 minutes and was implemented in ambient condition. After epoxidation pretreatment, C=C isomers of an unsaturated lipid species were mapped with targeted MS/MS (or MS^3) imaging. The MSI has the same spatial resolving power after mCPBA pretreatment as validated in **Fig. S35**.

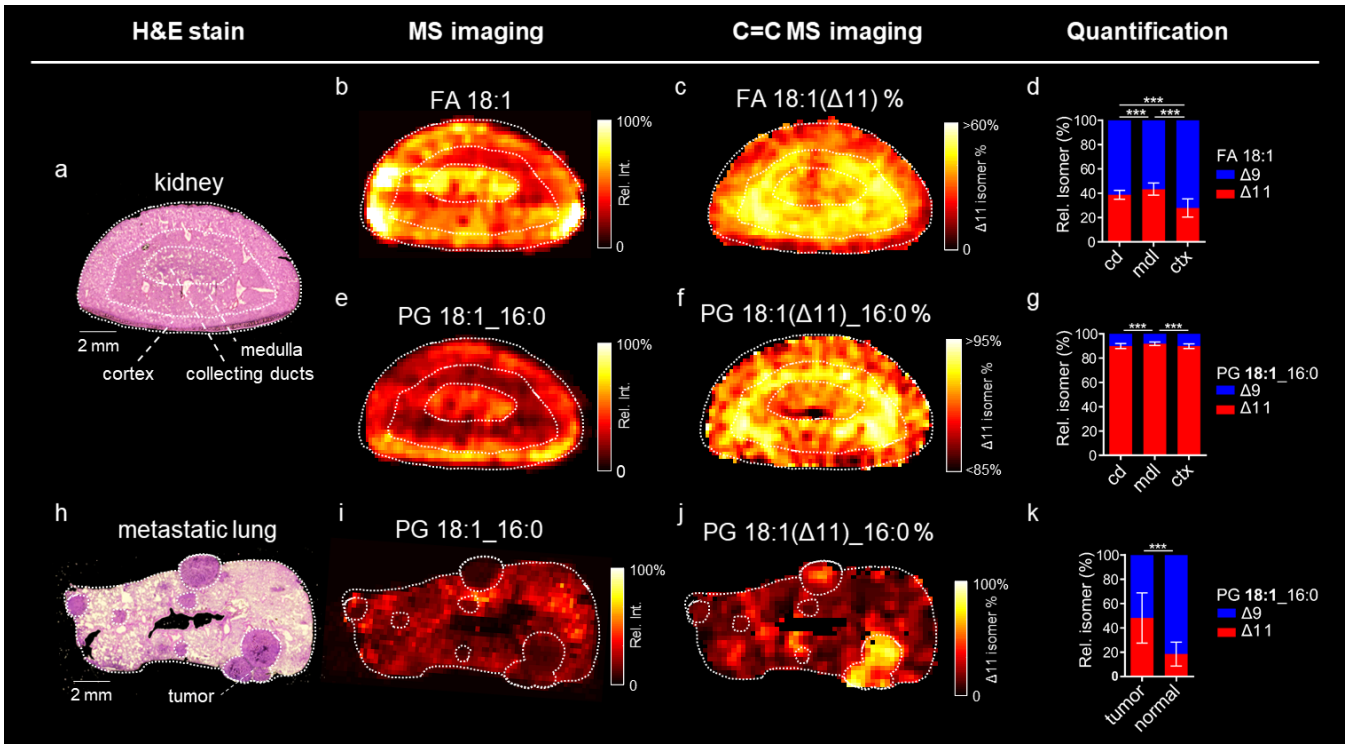


Figure 7. Mapping lipid C=C isomers in mouse tissues with MELDI-DESI-MSI. (a-f) C=C isomer imaging of a mouse kidney section. Distinct tissue regions are outlined by white dash lines, including cortex (ctx), medulla (mdl), and collecting ducts (cd) in the H&E staining image in **(a)**. Full-MS images of **(b)** FA 18:1 (*m/z* 281.25) and **(e)** PG 18:1_16:0 (*m/z* 747.52) are shown, and their corresponding C=C images of the Δ11 isomer are shown as fractional distribution image (FDI) of Δ11/(Δ9+Δ11) % in **(c)** and **(f)**, respectively. **(h-j)** C=C isomer imaging of a metastatic lung tissue. **(i)** Full-MS image of PG 18:1_16:0 and **(j)** the corresponding FDI of the Δ11 isomer. (**d, g, k)** Quantification of relative proportions of C=C isomers in each tissue region (*t* test, *** $P < 0.005$). Errors bars represent SD.

Using the mouse kidney sections for our first example of MELDI-DESI-MSI, as shown in **Fig. 7b**, the ion image of *m/z* 281.25, which represented the summed distributions of all FA 18:1 C=C isomers, was observed with a slightly lower intensity in the kidney medulla. In the adjacent section pretreated by mCPBA, the epoxidation product, epoxy-FA 18:1 (*m/z* 297.24), could be clearly observed in the full-MS spectrum (**Fig. S36b**). The on-tissue CID spectrum of epoxy-FA 18:1 clearly confirmed the presence of two C=C isomers with C=C positions at either Δ9 or Δ11 (**Fig. S36c**). Targeted MS/MS imaging of epoxy-FA 18:1 enabled the semi-quantitative molecular imaging of C=C isomers by fractional distribution image (FDI) of Δ9 and Δ11 isomers (**Fig. 7c**). Here, an enriched level of the Δ11 isomer, and conversely a decreased level of Δ9 isomer, was observed in the kidney medulla (**Fig. 7d**), which implied that the decreased level of total FA 18:1 in the kidney medulla might be the result of decreased Δ9 isomer. This highlighted that FDI images showed the changes in the ratio of diagnostic ion intensities rather than the actual local concentration of C=C isomers, while alteration of C=C isomer composition could be clearly reflected by the FDI images across the tissue section. The same approach was performed for PG 16:0_18:1 in the other set of serial kidney sections. Full-MS imaging of *m/z* 747.52 showed a relatively lower PG 16:0_18:1 abundance in the kidney medulla (**Fig. 7e**). The *in situ* MS^3 spectrum of epoxy-PG 18:1_16:0 (*m/z* 763.51) in the epoxidized section also showed a mixture of Δ9 and Δ11 isomers (**Fig. S36d**). Mapping the C=C isomers through FDI revealed the enrichment of the Δ11 isomer proportion in the kidney medulla (**Fig. 7f**). Interestingly, the overall fraction of Δ11 isomer in PG 18:1_16:0, calculated to be about 90%, was remarkably higher than that of FA 18:1 (**Fig. 7g**), implying that FA 18:1 (Δ11) isomer was preferably selected during synthesis of PG 18:1_16:0 in the mouse kidney

The power of MELDI-DESI-MSI allowed us to explore the tumor-associated alteration of C=C isomers. Such proposed idea was demonstrated in a metastatic lung tissue invaded by breast tumor cells (**Fig. 7h**). As shown in **Fig. 7i**, the tumor region could not be distinguished by the summed MSI of PG 18:1_16:0, later resolved as the mixture of two C=C isomers of Δ9 and Δ11 on the mCPBA-pretreated section (**Fig. S37d**). Intriguingly, MELDI-DESI-MSI revealed that the fraction of PG 16:0_18:1 Δ11 isomer was significantly elevated in the tumor cell regions (**Fig. 7j and 7k**), suggesting that a single pair of C=C positional isomers could be utilized to determine the tumor margin.

Conclusion

We concluded that MELDI is an easy-to-use and cost-effective derivatization strategy to pinpoint lipid C=C positions coupling with MS/MS analysis. To be specific, epoxidation with mCPBA gives several outstanding features, including (i) one-pot, rapid, catalyst-free, single-

step derivatization in organic solvents under mild conditions, (ii) high specificity to C=C bonds and appreciable yield of epoxide (**Table S1)**, (iii) inexpensive reagents (market price ~0.1 USD per gram of mCPBA powder, and (iv) high stability of epoxides in room temperature (**Fig. S11c**). With these features, MELDI can thus provide high sensitivity in identifying C=C isomers and is feasible for a broad range of lipid classes (**Fig. S37 and Table S13)**, suggesting an innovative way for studying lipid C=C isomers in biomedical research. In yet another perspective, we noted that the C=C isomer alterations in PG (**Fig. 7**) might be contributed by bis(monoacylglycero)phosphate (BMG), which is the *sn*-substitutional isomer of PG. Therefore, we believe that the combination of MELDI with other *sn*-substitutional isomer-resolving techniques, such as OzID and UVPD, will allow a more complete structural elucidation of unsaturated lipids. In the end, we would like to highlight that MELDI is of great potential as being integrated into versatile MS platforms for lipid C=C isomer analyses. Besides ESI-MS,[35] LC-MS, and DESI-MS, MELDI is readily applicable to many frequently used ionization sources, including MALDI, paper-spray ionization, and atmospheric pressure chemical ionization (APCI) (**Fig. S38-S40**), allowing many non-instrument-oriented lipid scientists to study lipid C=C isomers in a routine manner.

Lipidomics has been profoundly used as a mean for disease biomarker discovery.[1] An inevitable demand in lipidomics is to elucidate chemical structures of potential lipid biomarkers, which is challenging due to the complexity of lipid structural isomers. Recent advances in MS technology have become promising in providing detailed structural assignments of lipid C=C positions,[43] leading to a growing understanding of the disease-related alteration in C=C isomer compositions.[10,23,28] MELDI enabled us to such deep analysis of C=C isomers effectively. Using the SCD1-inhibited 3T3-L1 adipocytes as an example, we found that SCD1 acts as a regulator in maintaining cellular lipid homeostasis at the level of multiple C=C isomer compositions. As aberrant SCD1 activity along with disrupted lipid balance are associated with diabetes, obesity, fatty liver diseases, and cancers,[44] MELDI-LC-MS-tPRM analysis provides us a powerful way to gain further insights into how cellular lipidome is reprogrammed as a disease progresses. Moreover, coupling MELDI with DESI-MSI resolved the relative ratios of C=C isomers in a spatial manner, providing a new approach to determine the tumor margins. Therefore, we believe that, in the foreseeable future, MELDI will have a great impact to uncover the underlying lipid biochemistry by a deep mining of lipid C=C isomers in biological systems.

ASSOCIATED CONTENT

SUPPORTING INFORMATION

Materials and methods, the spectral data set of lipid C=C isomer standards, features of mCPBA epoxidation, data processing strategy in LC-MS-tPRM analysis, EpoxyFinder software package, the demonstration video as well as the method for for *in situ* derivatization using mCPBA, and other related contents are provided in Supporting Information.

AUTHOR INFORMATION

Corresponding Author

* Cheng-Chih Hsu: ccrhsu@ntu.edu.tw,

ORCID

Ting-Hao Kuo: 0000-0001-5130-0570
Cheng-Chih Hsu: 0000-0002-2892-5326

Author Contributions

T.-H. K. and C.-C. H. designed the experiments. T.-H. K. and H.-H. C. carried out all the MS experiments and analyzed the data. H.-Y. C. constructed the cell experiments. C.-W. L., T.-L. S., and M.-Y. W. designed and carried out the animal experiments and collected the clinical samples. C.-C. H. supervised the study. T.-H. K. and C.-C. H. wrote the paper.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

This research was supported by Ministry of Science and Technology (MOST), R.O.C. (Grant nos.: MOST 106-2113-M-002-013-MY2, 107-2321-B-001-038-, and 108-2636-M-002-008-), and Center for Emerging Materials and Advanced Devices, National Taiwan University (NTU) (Grant nos.: NTU-ERP-108L880116). T.-H. K. acknowledges the financial support from National Taiwan University, and Institute of Nuclear Energy Research, Atomic Energy Council, Executive Yuan, R.O.C. The instrument support from NTU Mass Spectrometry Platform was acknowledged. The technical assistance from Ying-Chen Huang, Li-En Lin, Kai-Hung Huang, and Dr. Qiang Lyu in C.-C. H. Lab, Ko-Chien Chen and Yu-Ju Wang in T.-L. S. Lab, and Bo-Rong Chen in M.-Y. W. Lab, was also acknowledged.

REFERENCES

1. K. Yang; X. Han, Lipidomics: Techniques, applications, and outcomes related to biomedical sciences. *Trends Biochem. Sci.* **2016,** *41* (11), 954-969.
2. S. J. Blanksby; T. W. Mitchell, Advances in mass spectrometry for lipidomics. *Annu. Rev. Anal. Chem.* **2010,** *3*, 433-65.
3. Y. H. Rustam; G. E. Reid, Analytical challenges and recent advances in mass spectrometry based lipidomics. *Anal. Chem.* **2018,** *90* (1), 374-397.
4. J. A. Bowden, et al., Harmonizing lipidomics: NIST interlaboratory comparison exercise for lipidomics using SRM 1950-metabolites in frozen human plasma. *J. Lipid Res.* **2017,** *58* (12), 2275-2288.
5. O. Quehenberger, et al., Lipidomics reveals a remarkable diversity of lipids in human plasma. *J. Lipid Res.* **2010,** *51* (11), 3299-305.
6. H. Guillou; D. Zadravec; P. G. Martin; A. Jacobsson, The key roles of elongases and desaturases in mammalian fatty acid metabolism: Insights from transgenic mice. *Prog. Lipid Res.* **2010,** *49* (2), 186-99.
7. P. E. Williams; D. R. Klein; S. M. Greer; J. S. Brodbelt, Pinpointing double bond and sn-positions in glycerophospholipids via hybrid 193 nm ultraviolet photodissociation (UVPD) mass spectrometry. *J. Am. Chem. Soc.* **2017,** *139* (44), 15681-15690.
8. D. R. Klein; J. S. Brodbelt, Structural characterization of phosphatidylcholines using 193 nm ultraviolet photodissociation mass spectrometry. *Anal. Chem.* **2017,** *89* (3), 1516-1522.
9. E. Ryan; C. Q. N. Nguyen; C. Shiea; G. E. Reid, Detailed structural characterization of sphingolipids via 193 nm ultraviolet

photodissociation and ultra high resolution tandem mass spectrometry. *J. Am. Soc. Mass Spectrom.* **2017,** *28* (7), 1406-1419.

10. D. R. Klein, et al., Desorption electrospray ionization coupled with ultraviolet photodissociation for characterization of phospholipid isomers in tissue sections. *Anal. Chem.* **2018,** *90* (17), 10100-10104.

11. T. Baba; J. L. Campbell; J. C. Le Blanc; P. R. Baker, Structural identification of triacylglycerol isomers using electron impact excitation of ions from organics (EIEIO). *J. Lipid Res.* **2016,** *57* (11), 2015-2027.

12. T. Baba; J. L. Campbell; J. C. Le Blanc; P. R. Baker, In-depth sphingomyelin characterization using electron impact excitation of ions from organics and mass spectrometry. *J. Lipid Res.* **2016,** *57* (5), 858-67.

13. M. C. Thomas, et al., Ozone-induced dissociation: Elucidation of double bond position within mass-selected lipid ions. *Anal. Chem.* **2008,** *80* (1), 303-11.

14. S. E. Hancock, et al., Mass spectrometry-directed structure elucidation and total synthesis of ultra-long chain (o-acyl)-omega-hydroxy fatty acids. *J. Lipid Res.* **2018,** *59* (8), 1510-1518.

15. M. R. L. Paine, et al., Mass spectrometry imaging with isomeric resolution enabled by ozone-induced dissociation. *Angew. Chem. Int. Ed. Engl.* **2018,** *57* (33), 10530-10534.

16. B. L. Poad, et al., High-pressure ozone-induced dissociation for lipid structure elucidation on fast chromatographic timescales. *Anal. Chem.* **2017,** *89* (7), 4223-4229.

17. N. Vu; J. Brown; K. Giles; Q. Zhang, Ozone-induced dissociation on a traveling wave high-resolution mass spectrometer for determination of double-bond position in lipids. *Rapid Commun. Mass Spectrom.* **2017,** *31* (17), 1415-1423.

18. B. L. J. Poad, et al., Online ozonolysis combined with ion mobility-mass spectrometry provides a new platform for lipid isomer analyses. *Anal. Chem.* **2018,** *90* (2), 1292-1300.

19. M. C. Thomas; T. W. Mitchell; S. J. Blanksby, Ozonolysis of phospholipid double bonds during electrospray ionization: A new tool for structure determination. *J. Am. Chem. Soc.* **2006,** *128* (1), 58-9.

20. M. C. Thomas, et al., Elucidation of double bond position in unsaturated lipids by ozone electrospray ionization mass spectrometry. *Anal. Chem.* **2007,** *79* (13), 5013-22.

21. R. A. Harris, et al., Determining double bond position in lipids using online ozonolysis coupled to liquid chromatography and ion mobility-mass spectrometry. *Anal. Chem.* **2018,** *90* (3), 1915-1924.

22. X. Ma; Y. Xia, Pinpointing double bonds in lipids by Paterno-Buchi reactions and mass spectrometry. *Angew. Chem. Int. Ed. Engl.* **2014,** *53* (10), 2592-6.

23. X. Ma, et al., Identification and quantitation of lipid C=C location isomers: A shotgun lipidomics approach enabled by photochemical reaction. *Proc. Natl. Acad. Sci. U. S. A.* **2016,** *113* (10), 2573-8.

24. X. Ma, et al., Photochemical tagging for quantitation of unsaturated fatty acids by mass spectrometry. *Anal. Chem.* **2016,** *88* (18), 8931-5.

25. J. Ren; E. T. Franklin; Y. Xia, Uncovering structural diversity of unsaturated fatty acyls in cholesteryl esters via photochemical reaction and tandem mass spectrometry. *J. Am. Soc. Mass Spectrom.* **2017,** *28* (7), 1432-1441.

26. F. Tang, et al., Rapid in situ profiling of lipid c horizontal linec location isomers in tissue using ambient mass spectrometry with photochemical reactions. *Anal. Chem.* **2018,** *90* (9), 5612-5619.

27. A. Bednarik; S. Bolsker; J. Soltwisch; K. Dreisewerd, An on-tissue Paterno-Buchi reaction for localization of carbon-carbon double bonds in phospholipids and glycolipids by matrix-assisted laser-desorption-ionization mass-spectrometry imaging. *Angew. Chem. Int. Ed. Engl.* **2018,** *57* (37), 12092-12096.

28. W. Zhang, et al., Online photochemical derivatization enables comprehensive mass spectrometric analysis of unsaturated phospholipid isomers. *Nat. Commun.* **2019,** *10* (1), 79.

29. J. Deng, et al., Coupling Paterno-Buchi reaction with surface-coated probe nanoelectrospray ionization mass spectrometry for in vivo and microscale profiling of lipid C=C location isomers in complex biological tissues. *Anal. Chem.* **2019,** *91* (7), 4592-4599.

30. Y. Zhao, et al., Identification and quantitation of C=C location isomers of unsaturated fatty acids by epoxidation reaction and tandem mass spectrometry. *Anal. Chem.* **2017,** *89* (19), 10270-10278.

31. X. Zhao, et al., Rapid analysis of unsaturated fatty acids on paper-based analytical devices via online epoxidation and ambient mass spectrometry. *Anal. Chem.* **2018,** *90* (3), 2070-2078.

32. W. Cao, et al., Locating carbon-carbon double bonds in unsaturated phospholipids by epoxidation reaction and tandem mass spectrometry. *Anal. Chem.* **2018,** *90* (17), 10286-10292.

33. K. Yang; B. G. Dilthey; R. W. Gross, Identification and quantitation of fatty acid double bond positional isomers: A shotgun lipidomics approach using charge-switch derivatization. *Anal. Chem.* **2013,** *85* (20), 9742-50.

34. H. Hussain, et al., meta-chloroperbenzoic acid (mCPBA): A versatile reagent in organic synthesis. *RSC Adv.* **2014,** *4* (25), 12882-12917.

35. Y. Feng; B. Chen; Q. Yu; L. Li, Identification of double bond position isomers in unsaturated lipids by m-CPBA epoxidation and mass spectrometry fragmentation. *Anal. Chem.* **2019,** *91* (3), 1791-1795.

36. N. Rauniyar, Parallel reaction monitoring: A targeted experiment performed using high resolution and high mass accuracy mass spectrometry. *Int. J. Mol. Sci.* **2015,** *16* (12), 28566-81.

37. J. Ecker; M. Scherer; G. Schmitz; G. Liebisch, A rapid GC-MS method for quantification of positional and geometric isomers of fatty acid methyl esters. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2012,** *897*, 98-104.

38. J. C. Ralston; D. M. Mutch, Scd1 inhibition during 3T3-L1 adipocyte differentiation remodels triacylglycerol, diacylglycerol and phospholipid fatty acid composition. *Prostaglandins Leukot. Essent. Fatty Acids* **2015,** *98*, 29-37.

39. A. M. Porcari, et al., Multicenter study using desorption-electrospray-ionization-mass-spectrometry imaging for breast-cancer diagnosis. *Anal. Chem.* **2018,** *90* (19), 11324-11332.

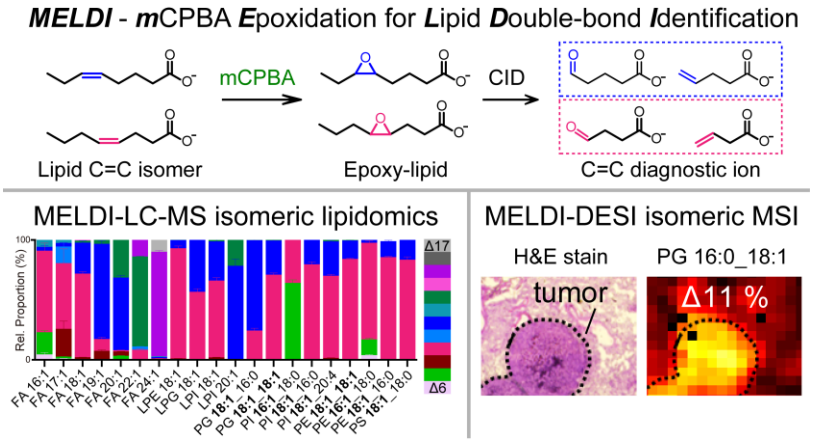
40. S. R. Ellis, et al., Automated, parallel mass spectrometry imaging and structural identification of lipids. *Nat. Methods* **2018,** *15* (7), 515-518.

41. Z. Takats; J. M. Wiseman; B. Gologan; R. G. Cooks, Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. *Science* **2004,** *306* (5695), 471-3.

42. S. A. Schwartz; M. L. Reyzer; R. M. Caprioli, Direct tissue analysis using matrix-assisted laser desorption/ionization mass spectrometry: Practical aspects of sample preparation. *J. Mass Spectrom.* **2003,** *38* (7), 699-708.

43. T. Porta Siegel; K. Ekroos; S. R. Ellis, Reshaping lipid biochemistry by pushing barriers in structural lipidomics. *Angew. Chem. Int. Ed. Engl.* **2019**, doi: 10.1002/anie.201812698.

44. R. A. Igal, Stearoyl coa desaturase-1: New insights into a central regulator of cancer metabolism. *Biochim. Biophys. Acta.* **2016,** *1861* (12 Pt A), 1865-1880.


MELDI - mCPBA Epoxidation for Lipid Double-bond Identification
mCPBA
CID
Lipid C=C isomer
Epoxy-lipid
C=C diagnostic ion
MELDI-LC-MS isomeric lipidomics
Rel. Proportion (%)
100
0
Δ17
Δ6
FA 16:1
FA 17:1
FA 18:1
FA 19:1
FA 20:1
FA 22:1
FA 24:1
LPE 18:1
LPG 18:1
LPI 18:1
LPI 20:1
PG 18:1_16:0
PG 18:1_18:1
PI 16:1_18:0
PI 18:1_18:0
PI 18:1_16:0
PE 18:1_20:4
PE 16:1_18:1
PE 18:1_18:0
PE 18:1_16:0
PS 18:1_18:0
MELDI-DESI isomeric MSI
H&E stain
PG 16:0_18:1
tumor
Δ11 %